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Effects of NO_2 on Chronic Bronchitics

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The acute influence of NO_2 on mechanics of breathing and respiratory gas exchange was investigated in a total of 111 subjects, aged 25 to 74 years, with chronic nonspecific lung disease (CNSLD). They breathed NO_2–air mixtures containing 0.5 to 8.0 ppm NO_2 for up to 15 to 60 min. Additionally in nine subjects the protective action of atropine, meclastine, and orciprenaline was investigated.

While the alveolar PO_2 remained nearly constant during inhalation of 5 and 4 ppm NO_2, a significant decrease of the arterial PO_2 and a corresponding increase of the arterial to alveolar PO_2 gradients occurred. Inhalation of 2 ppm NO_2 had not such an effect.

Inhalation of NO_2 at concentrations down to 1.5 ppm resulted in a significant increase of airway resistance. Lower concentrations had no significant effect.

Prolongation of the exposure period from 15 to 60 min at a NO_2 concentration of 5 ppm did not result in a more pronounced disturbance of the respiratory gas exchange for oxygen beyond the extent observed after exposure to 5 ppm NO_2 for 15 min.

Meclastine, in comparison with orciprenaline and atropine, showed a pronounced protective effect on the negative impact of NO_2 on respiratory gas exchange and airway resistance.

It is concluded that NO_2 may act by release of histamine, causing a bronchiolar, alveolar, and interstitial edema, thus differing from irritant air pollutants like SO_2, where reflex bronchoconstriction causes in some bronchitics dramatic increases of airway resistance at similar low concentrations.

Introduction

Chronic nonspecific lung diseases are an important cause of disability and untimely death in all industrialized countries; this seems to be related to occupational and environmental pollution.

Existing data from experimental animal studies with oxidizing air pollutants like NO_2 have shown that chronic inhalation leads to serious morphological lesions especially in the lung periphery (*1*) whereas SO_2, for example, does not seem to have such effects or only at higher concentrations (*2, 3*).

Human experimental exposure studies are restricted to short-term exposures in the range of MAK concentrations and below or of concentrations observed in ambient air because of ethical considerations, and it is discussed whether such experiments can be performed on the more susceptible subjects like asthmatics or bronchitics who need special protection by safe standards. [The German MAK value (maximal allowable concentration for occupational exposure) mentioned in this paper corresponds to the TLV, i.e., 5 ppm for NO_2.]

Although there is a quite general agreement that changes of lung function will occur when inhaling this pollutant at concentrations equal to or exceeding MAK values, some points request further examination, namely, determination of the concentration range where first changes of pulmonary function are to be observed ("no-effect level"); clarification whether changes are caused by reflectory bronchoconstriction or effected by mediators like histamine; estimation from the results of these acute experiments the possibility of a risk of the development of chronic respiratory disease.

It has been the objective of the studies described here (*4, 5*) to support to the clarification of some of the aspects mentioned above. For this purpose, the acute influence of NO_2 on mechanics of breathing and respiratory gas exchange was investigated in subjects with chronic, nonspecific lung disease (CNSLD). They breathed NO_2–air mixtures from gas tight bags containing 0.5 to 8.0 ppm NO_2 for up to 5 and up to 60 min. Additionally in nine subjects the protective action of atropine, meclastine, and orciprenaline was investigated.

Methods and Subjects

A total of 116 patients, aged 25 to 74 years, suffering from chronic, nonspecific lung disease were

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investigated. These patients were admitted to the hospital because of an exacerbation of their disease. During the time of investigation their status had bettered and they did not suffer from severe airway obstruction or hypoxemia.

Lung function parameters of interest were those for the respiratory gas exchange for oxygen and carbon dioxide (PaO_2, PAO_2, $PaCO_2$, $PACO_2$, and pHa), airway resistance (R_{aw}) measured as total airway resistance from the extreme pressure points of the pressure/flow diagram (*6*) and thoracic gas volume (TGV) to indicate functional residual capacity (FRC) where R_t was measured. Phase shift caused by the Fleisch pneumotachograph when breathing at higher frequencies was avoided by having the subjects breathe at normal frequencies. Measurements of R_t has proved to be a sensitive indicator for changes in lung function associated with gaseous pollutants at low concentrations (*7–10*). (Dynamic manoeuvres like FEV_1 or V_{50} probably are less suited for such investigations because they themselves—especially in bronchitics and asthmatics—may influence the results.)

Continuous measurement of PAO_2 and $PACO_2$ (as measured in bypass at the mouthpiece) was used for control of the steady state necessary for arterial PO_2 and PCO_2 analysis and for calculation of the alveolar-to-arterial PO_2 and PCO_2 gradients $AaDO_2$ and $aADCO_2$. The blood samples were taken from hyperemized ear lobe blood in sitting position. Hyperemized blood from the ear lobe was considered to be representative for arterial blood according to preparatory studies comparing PO_2 in blood simultaneously obtained by arterial puncture and in micro samples from the hyperemized ear lobe (*11*). The usability of this method was further approved by more recent investigations of PO_2 measurement via the skin by means of a continuously measuring platinum electrode, where the PO_2 follows immediately the changes in alveolar PO_2 thus giving exact the features of respiratory gas exchange (*16*).

The instruments used were a respiratory mass spectrometer (Varian MAT), platinum and glass electrodes (Eschweiler), and a constant volume body plethysmograph (own construction analogous to the Siemens body box with electronic compensation of differences in temperature and water vapor between inhaled and expired air allowing breathing at normal frequencies (*12*).

Measurement of lung function parameters was

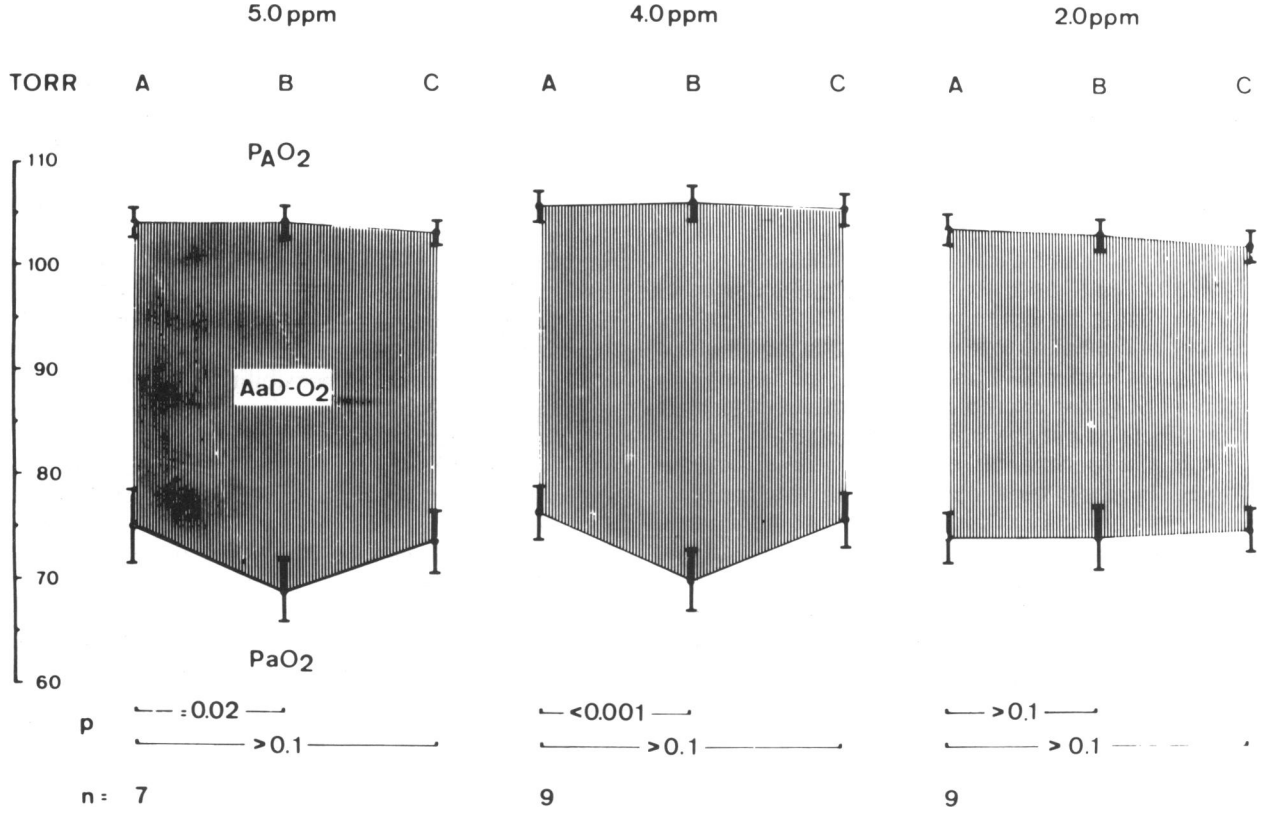

FIGURE 1. Mean values and standard deviations of PAO_2 and PaO_2 (*A*) before, (*B*) at the end, and (*C*) after inhalation of 5, 4, and 2 ppm NO_2 for 15 min.

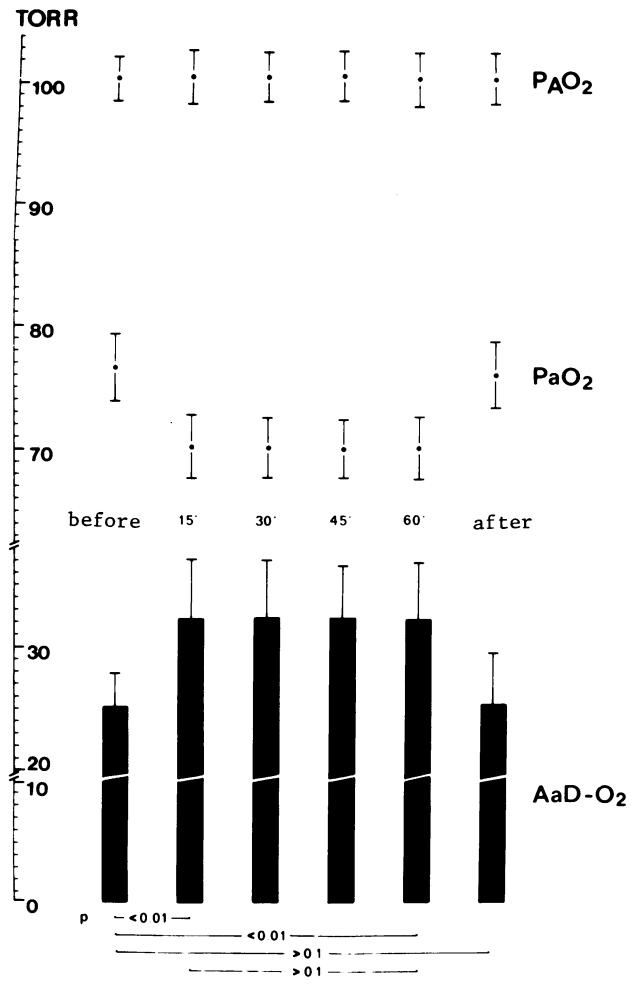

FIGURE 2. Mean values and standard deviations of alveolar and arterial oxygen partial pressures (PAO_2 and PaO_2) and the alveolar to arterial oxygen gradients ($AaDO_2$) before, during, and after a 60 min exposure to NO_2 concentrations of 5 ppm (n = 14).

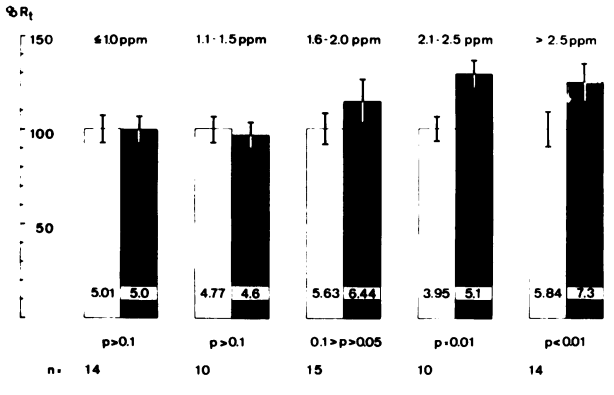

FIGURE 3. Increase in total airway resistance (R_t) (mean values and standard deviations) before and after inhalation of NO_2 at different concentrations (initial value = 100%).

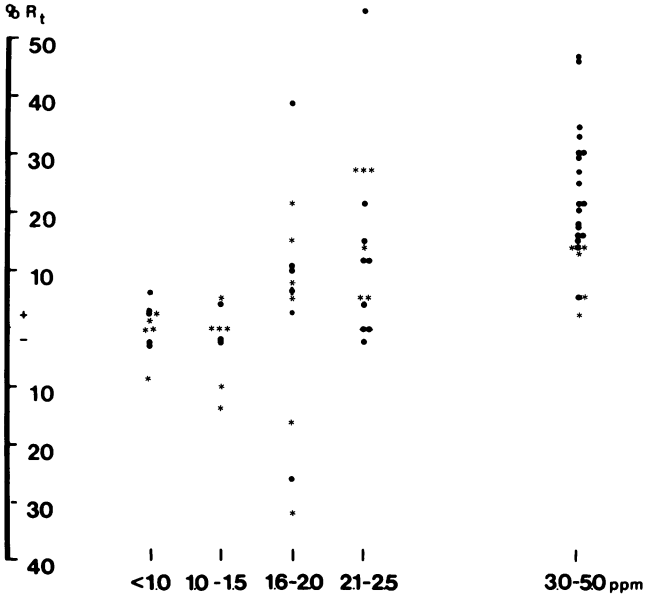

FIGURE 4. Dependence of the increase in relative airway resistance (R_t) upon the concentration (r = 0.542): (*) smokers; (●) nonsmokers.

made at the end of the prephase, after exposure to the different NO_2–air mixtures (1.0–8.0 ppm) for different times (up to 5 and up to 60 min) and within 1 hr after termination of exposure.

The protective action of atropine (0.75 mg SC), meclastine (2.68 mg IV) and orciprenaline (1.5 mg by inhalation) was tested as described previously (*5*).

Analysis of NO_2 was made by using the colorimetric Saltzman method (*13*).

The statistical evaluation was made with the aid of Wilcoxon's ranking method of pair differences (*14, 15*) where the null hypothesis (= no effect) was subjected to a one-sided test.

Results

While the alveolar PO_2 remained nearly constant during inhalation of 5 and 4 ppm NO_2 (15 min), a significant decrease of the arterial PO_2 and a corresponding increase of the arterial to alveolar PO_2 gradients occurred. Inhalation of 2 ppm NO_2 had no such effect (Fig. 1).

A prolongation of the exposure period from 15 to 60 min at a NO_2 concentration of 5 ppm did not result in a more pronounced disturbance of the respiratory gas exchange for oxygen beyond the extent observed after exposure to 5 ppm NO_2 for 15 min (Fig. 2).

Inhalation of NO_2 concentrations down to 1.5 ppm (30 breaths ~ 5 min) resulted in a significant increase of airway resistance. Lower concentra-

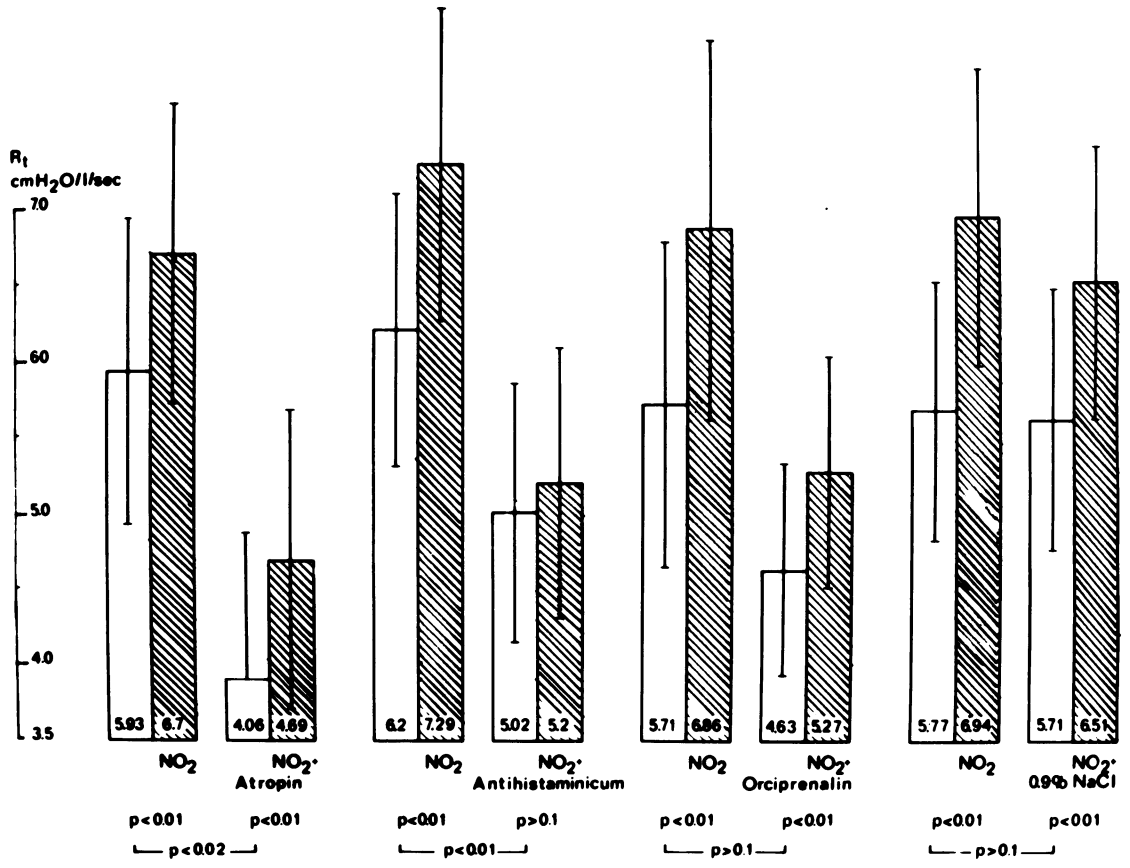

FIGURE 5. Mean values and standard deviations of total airway resistance R_t in 14 subjects with chronic bronchitis (left in each pair of columns) before and (right) after inhalation of 5 ppm NO_2 for up to 5 min without and with atropine, meclastine, orciprenaline, and NaCl as control.

tions had no significant effect (Figs. 3 and 4).

Meclastine, when compared with orciprenaline and atropine, showed a protective effect (Fig. 5) on the negative impact of NO_2 on respiratory gas exchange (30 breaths ~ 5 min) and airway resistance.

Discussion

The results indicate that short-term exposures to NO_2 may have discrete effects on human lung function. At NO_2 concentrations in the MAK range, the effect on respiratory gas exchange is relatively uniform: the consistent PaO_2 decrease of about 8 mm Hg indicates that irritant gases like NO_2 in low concentrations cause a uniform reaction in the lung. This is supported by a similarly uniform increase of airway resistance even in previously compromised subjects, where, corresponding to healthy subjects (*9, 10*), R_{aw} increases between 0.5 and 2.0 cm H_2O/(liter sec) may generally be observed.

After acute exposure to NO_2 airway resistance has shown to increase at concentrations above 1.5 ppm. In healthy subjects a similar increase of R_{aw} at NO_2 concentrations of 2.5 ppm was shown (*7*).

There is some indication that histamine release might play a role in triggering the effects on lung function observed after inhalation of NO_2. Thomas (*17*) has shown degranulation of mast cells in rat lung tissue after acute exposure to low NO_2 concentrations (0.5–1.0 ppm). In human subjects exposed to 5–8 ppm NO_2 for up to 5 min, a marked protective action of a histamine-suppressing agent (meclastine) was demonstrated; atropine or β-stimulating agents did not have such an effect (*5*). However, in preliminary studies with quantitative estimation of the histamine content of plasma before, during, and after inhalation of a combination of NO_2, O_3, and SO_2 for 2 hr at MAK concentrations, a well defined histamine release was demonstrated only in one half of the subjects (*18*).

As mentioned above, the experimental lung function studies in humans demonstrate that the functional response to the inhalation of NO_2 is quite similar in all subjects. Additionally, these very uni-

form and discrete changes, especially those of the airway resistance, suggest that the observed effects are probably due to formation of microedema of the bronchial or interstitial epithelium by histamine liberation and not by reflectory bronchoconstriction. However, histamine could also directly increase the tonus of the peripheral muscles, not being influenced by the nervus vagus, with disturbance of the ventilation/perfusion ratios and consecutive decrease of PaO_2 (*19*).

Reflectory bronchoconstriction after vagal stimulation probably would lead to a more inhomogeneous reaction of the bronchial tree with less uniform reactions of airway resistance in the different subjects. Reflex bronchoconstriction may be true for SO_2 inhalation, where the possibility of a vagal stimulated bronchoconstriction is discussed (*8*) and where at similar low concentrations in patients with obstructive lung disease dramatic increases of R_{aw} are likely to occur (*20*). The more pronounced increase of airway resistance after inhalation of NO_2 alone or $NO_2 + SO_2 + O_3$ in combination with a bronchoconstricting agent like carbachol or acetylcholine is probably due to an increased reactivity of sensory receptors caused by air pollutants and thus a vagal reaction (*10, 21*).

An estimation of the risk of the development of chronic respiratory disease may be drawn from experimental animal studies: e.g., after short-term exposure to 5 ppm NO_2, a configuration change occurs in lung collagen and elastin. This denaturation of collagen and elastin was shown to be reversible when the animals were sacrificed 24 hr after termination of exposure (*22*). An exposure of rabbits to low concentrations of NO_2 (0.255 ppm) for 4 hr/day for 6 days, however, showed irreversible structural changes in lung collagen as determined by electron microscopy (*23*). Possibly owing to increased collagen and elastin catabolism in the lung, the hydroxyproline excretion was elevated in workers chronically exposed to NO_2 levels of 0.4–2.7 ppm (*24*) and in experimental animals after long-term inhalation of concentrations slightly above 2.5 ppm (*25*).

A crosslinking of collagen and elastin fibers observed in animals after short-term exposure may be an important fact in estimating part of the risk of development of chronic lung disease: this resembles an accelerated aging process, as normally collagen and elastin become macromolecules by crosslinking in aging processes. Equally important is the observation of a delayed maturation of the rat lung in an environment containing nitrogen dioxide (*26*). More recent findings indicate that exposures to NO_2 at low concentrations will cause a significant increase in the mortality of mice challenged with streptococcus aerosol after exposure to the pollutant. The same effect was seen at even lower pollutant levels when mixtures of NO_2 and O_3 at concentrations frequently found in ambient air were used (*27, 28*).

Chronic exposure to NO_2 leads to morphological changes in rats such as terminal bronchiolar hypertrophy, loss of cilia, desquamation of alveolar cells and thickening of alveolar septa at NO_2 concentrations of 2.0 ppm this being an index for preemphysematous changes in the lung (*1*). Additionally, long-term as well as short-term exposure to NO_2 leads to changes in the alveolar cell populations; after chronic exposure to concentrations of 2 ppm NO_2 pneumocyte I type cells were gradually replaced by the more cuboidal type II pneumocytes (*29–31*).

Although the carcinogenic or cocarcinogenic effects of NO_2 have not been proved to date, nitrosamine formation is also a potential hazard (*32, 33*).

Conclusions

In considering the concentrations of NO_2 at which adverse effects in humans first appear with ambient air concentrations, there is relatively little room for a sufficiently great "safety factor" when considering ambient air standards for NO_2.

A number of studies based on the use of lung function parameters as indicators for short-term effects have demonstrated that the MAK value for NO_2 is clearly above the level at which significant changes in lung function occur. It is concluded that NO_2 may act primarily by release of histamine, causing bronchiolar, alveolar, and interstitial edema, thus differing from irritant air pollutants like SO_2, where reflex bronchoconstriction is considered to cause dramatic increases of airway resistance in some bronchitics at similar low concentrations (*20*).

Increased susceptibility to infectious agents of bronchitics and asthmatics, demonstrated in animals (*26*), combination with an increased irritability of the bronchial system after short-term exposures to NO_2, as indicated by the study of Orehek et al. (*20*), and adverse effects on lung function, as discussed in this paper, may be a potential hazard; this has to be taken into consideration, when setting a short-term standard for NO_2.

REFERENCES

1. Freeman, G., Crane, S. C., Stephens, R. I., and Furiosi, N. I. Lesions of the lungs of rats continuously exposed to two parts per million of nitrogen dioxide. Arch. Environ. Health 17: 181 (1968).
2. Islam, M. S., Oellig, W.-P., and Weller, W. Respiration damage caused by long-term inhalation of high concentration of sulfur dioxide in dogs. Res. Exptl. Med. 171: 211 (1977).

3. Reid, L. An experimental study of hypersecretion of mucus in the bronchial tree. Brit. J. Exptl. Pathol. 44: 437 (1963).
4. Nieding, G. von, et al. Grenzwertbestimmung der akuten NO_2-Wirkung auf den respiratorischen Gasaustausch und die Atemwegswiderstände des chronisch lungenkranken Menschen. Int. Arch. Arbeitsmed. 27: 338 (1971).
5. Nieding, G. von, and Krekeler, H. Pharmakologische Beeinflussung der akuten NO_2-Wirkung auf die Lungenfunktion von Gesunden und Kranken mit einer chronischen Bronchitis. Int. Arch. Arbeitsmed. 29: 55 (1971).
6. Nolte, D., Reiff, E., and Ulmer, W. T. Die Ganzkörperplethysmographie. Respiration 25: 14 (1968).
7. Beil, M., and Ulmer, W. T. Wirkung von NO_2 im MAK-Bereich auf Atemmechanik und bronchiale Acetylcholinempfindlichkeit. Int. Arch. Occup. Environ. Health 38: 31 (1976).
8. Islam, M. S., Vastag, E., and Ulmer, W. T. Sulphur-dioxide induced bronchial hyperreactivity against acetylcholine.
9. Nieding, G. von, et al. Akute Wirkung von 5 ppm NO_2 auf die Lungen- und Kreislauffunktion des gesunden Menschen. Int. Arch. Arbeitsmed. 27: 234 (1970).
10. Nieding, G. von, et al. Zur akuten Wirkung von Ozon auf die Lungenfunktion des Menschen. VDJ-Ber. 270: 123 (1977).
11. Krekeler, H., Nieding, G. von, Liese, W., and Muysers, K. Sauerstoffpartialdruck im arteriellen Blut und im Kapillarblut des hyperämisierten Ohrläppchens in Norm-, Hyper- und Hypoxie. Pneumonolgie 146: 34 (1971).
12. Smidt, U., Muysers, K., and Buchheim, W. Electronic compensation of differences in temperature and water vapour between in- and expired air and other signal handling in body plethysmography. Progr. Respir., 4: 39 (1969).
13. Saltzman, B. E. Colorimetric microdetermination of nitrogen dioxide in the atmosphere. Anal. Chem. 26: 1949 (1954).
14. Wilcoxon, F. Individual comparisons by ranking methods. Biometrics 1: 80 (1945).
15. Sachs, L. Statistische Auswertungsmethoden. Springer Verlag, Berlin-Heidelberg-New York, 1969.
16. Löllgen, H., and Nieding, G. von. Kontinuierliche Messung der alveolo-arteriellen Sauerstoffpartialdruckdifferenz. Paper presented at 84th Meeting of the Deutsche Gesellschaft für Innere Medizin, Wiesbaden, April 1978, in press.
17. Thomas, H. V., Müller, P. K., and Wright, R. Response of rat lung mast cells to nitrogen dioxide inhalation. J. Air. Pollution Contr. Assoc. 17: 33 (1967).
18. Wagner, H. M., et al. Biochemical effects after short-term exposure of humans to NO_2, O_3 and SO_2 at MAK-concentrations. In preparation.
19. Lanser, K., Islam, M. S., and Ulmer, W. T. Untersuchungen zur Kontrolle der Ventilationsdurchblutungsrelation. Verh. Deut. Ges. Inn. Med. 80: 894 (1974).
20. Ulmer, W. T., and Islam, M. S. Auswirkungen akuter SO_2-Expositionen beim Menschen und Tier. VDI Rept. 314: 136 (1978).
21. Orehek, J., Massari, P., Gayrard, P., Grimaud, C., and Charpin, J. Effect of short-term, low-level nitrogen dioxide exposure on bronchial sensitivity of asthmatic patients. J. Clin. Invest. 57: 301 (1976).
22. Buell, G. C., Tokiwa, Y., and Müller, P. K.: Lung collagen and elastin denaturation in vivo following inhalation of nitrogen dioxide. Paper presented at the 59th Air Pollution Control Association Meeting, San Francisco, June 1966, paper no. 66.
23. Müller, P. K., Hitchcock, M. Air quality criteria—toxicological appraisal for oxidants, nitrogen oxides, and hydrocarbons. J. Air Pollution Contr. Assoc. 19: 670 (1969).
24. Kosmider, S., Ludyga, K., Misiewicz, A., Droździ, M., and Sagan, J. Experimentelle und klinische Untersuchungen über emphysembildende Wirkungen der Stickoxide. Zbl. Arbeitsmed. Arbeitsschutz 12: 362 (1972).
25. Lindner, J., and Zorn, H. Das Hydroxyprolin im Urin als Mass für die NO_2-bedingte Kollagenschädigung der Atmungsorgane. Staub-Reinhalt. Luft 35: 166 (1975); Arch. Environ. Health 17: 181 (1968).
26. Freeman, G., Juhos, L. T., Furiosi, N. J., Mussende, R., and Weiss, T. A. Delayed maturation of rat lung in an environment containing nitrogen dioxide. Am. Rev. Resp. Dis. 110: 754 (1974).
27. Ehrlich, R. Effect of nitrogen dioxide on resistance to respiratory infection. Bacteriol. Rev. 30: 604 (1966).
28. Ehrlich, R., et al. Health effects of short-term inhalation of nitrogen dioxide and ozone mixtures. Environ. Res. 14: 223 (1977).
29. Sherwin, R. P., Dibble, J., and Weiner, J. Alveolar wall cells of the guinea pig, increase in response to 2 ppm nitrogen dioxide. Arch. Environ. Health 24: 43 (1972).
30. Stephens, R. J., Freeman, G., and Evans, J. M. Connective tissue changes in lungs of rats exposed to NO_2. Paper presented at 10th Annual Hanford Biology Symposium, Richland, Washington, June 2–5, 1970.
31. Stephens, R. J., Freeman, G., and Evans, M. J. Ultrastructural changes in connective tissue in lungs of rats exposed to NO_2. Arch. Int. Med. 127: 873 (1971).
32. Druckrey, H., Preussmann, R. Zur Entstehung carcinogener Nitrosamine am Beispiel des Tabakrauches. Naturwiss. 49: 498 (1962).
33. Sander, J., Bürkle, G., Slohe, L., and Aeikens, B. Untersuchungen *in vitro* über die Möglichkeit einer Bildung cancerogener Nitrosamine im Magen. Arzneimittelforschung (Drug.-Res.) 21: 411 (1971).